BIOLOGICAL MONITORING OF 1,3-DICHLOROPROPENE

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To demonstrate the excretion patterns of the metabolite of dichloropropene (DCP), N-acetyl-S-cis-3-chloroprop-2-enyl)-cysteine (3C-NAC), three applicators were studied. Urines were collected every 2-16 hours for 4 days, while air concentrations of DCP were determined by EC-GC from personal air monitoring charcoal collection tubes. 3C-NAC in urine was extracted, derivatized and measured on capillary gas chromatograph-mass spectrometry by monitoring the two abundant ions 117 and 176 m/e. Air concentrations of DCP ranged from 0.0-46.5 mg/m^3, with 8-hour TWA values ranging from 0.12-9.81 mg/m^3. Urine concentrations of 3C-NAC ranged 0.9-17.1 mg/L and urine excretion rates varied from 4-861 ug/hr. Exposure periods ranged 4-8 hours/day. Excretion of peak concentrations of metabolite followed peak exposure by variable periods of time (6-16 hours). Cumulative daily exposure correlated with cumulative excretion as indicated in previous work.

1,3-Dichloropropene (DCP) is a nematocide applied as a pre-harvest soil fumigant. It contains both cis- and trans- isomers (Telone II, 98%) and other chloropropenes and propanes. Older mixtures of less purity formulated by other companies are no longer marketed. DCP has a boiling point around 110 degrees and a vapor pressure of 28 mmHg. At 3 ppm, a sweet chlorform-like odor is detectable by smell. Liquid DCP is injected into the soil by being pumped through steel shanks which travel a few inches beneath the soil while being pulled by a tractor. Dichloropropene escapes from the soil via diffusion and evaporation, as well as volatilization of the liquid dripping from the injector shanks as it is removed from the soil at the end of each row. Applicators and loaders are exposed to this chlorinated hydrocarbon during the loading, maintenance, and application process (1,2).

The use of DCP has increased in recent years since ethylene dibromide (EDB) and 1,2-dibromo-3-chloropropane (DBCP) were banned for pesticide applications. The chemical structure of DCP is related to these two carcinogens and reproductive toxins as well as

to vinyl chloride. Animal studies with DCP have shown pulmonary hemorrhage, pulmonary edema, CNS depression and ataxia following inhalation of greater than 400 ppm for short periods of time. Direct dermal erythema and necrosis have been demonstrated upon acute skin exposure. Oral LD_5 studies (about 400 mg/kg) have shown that DCP produces liver and kidney necrosis (3). Animals exposed to 4.54 mg/m^3 (13 ppm) seven hours/day for 125-130 days showed a cloudy swelling of the renal tubular epithelium, which proved to be reversible. There was no significant adverse effect to the liver. DCP has been shown to be directly mutagenic (without microsomal activation) and also activated to a mutagen (with microsomes) (5,6). One study demonstrates that the mutagenic activity may be due to a degradation product (7). The NTP (NCI) carcinogenicity study of DCP has shown the bladder and gastric cancers are produced after oral gavage administration. Recent inhalation carcinogenicity studies have been completed in the rat and mouse and show some pulmonary tumors in the mouse.

There are few reports of human toxicity. Maddy et al. (1) demonstrated air concentrations near workers up to 4 ppm during the loading and application process in 1979. Statistics from the California Department of Food and Agriculture indicate only occasional reports of skin and eye irritation (8), though toxic effects to the kidneys and liver would not routinely be monitored. Other reports of human toxicity are from ingestions or accidents. In 1975, two firemen using a protective breathing apparatus were exposed to dichloropropene when the apparatus failed. One fireman became unconscious. Also, in 1975 a truck spilled 1200 gallons of dichloropropene and exposed 46 individuals. Twenty-one persons were hospitalized complaining of respiratory distress, dizziness, chest tightness, and several were found to have minor elevations of liver enzymes released into their serum. Several of these individuals, as well as one worker in a separate incident exposed to a one pint spill, complained of headaches and exhibited personality changes several months after the initial contact (9). Of most serious concern is the coincidence of the report of three individuals who had single exposures to dichloropropene and within seven years developed leukemia or histiocytic lymphoma (10). The rare nature of the two cases of histiocytic lymphoma and the rare exposure to DCP raises some concern.

In 1971, Hudson et al (11), demonstrated that ^{14}C-DCP oral gavage in rats led to an excretion of 82% of this radiolabelled activity in the urine. Small amounts were also excreted in the feces and in the expired breath. Only 61% of the trans-DCP radiolabelled activity was excreted in the urine. Following this, Climie et al (12) demonstrated that 77% of an oral dose of ^{14}C-cis-1,3-DCP was excreted in the urine as a thiol conjugate of N-acetyl cysteine (3C-NAC, Figure 1). In 1982-84, our laboratory synthesized this product as an unlabelled compound and an analogous conjugate from 2,3-DCP for use as an internal standard, developed extraction and gas chromatography-mass spectrometry (GC-MS) procedures for the determination of this metabolite in human urine (1). By obtaining spot and 24 hour urine collections from DCP applicators, we were able to show that milligram quantities of this metabolite were

excreted daily in these workers (see Table I). Those results showed that there was a rough correlation between the concentration x time product for air concentrations with the excretion of 3C-NAC in the urine (1).

Table I. 3C-NAC Metabolite Excretion - 1984

Subject (#)	Exposure (min)	Air DCP (TWA, mg/m^3)	Urine Vol (mL)	Excretion 3C-NAC/24 hr (mg)
1	150	1.50	1200	0.96
2	370	0.59	1830	5.46
3	277	1.64	1395	8.01
4	364	1.86	865	8.95
5	175	1.61	720	3.68

Spot urines (n=8) 5.2 - 27.0 ug/mL

The objective of the current study was to perform preliminary studies as a basis for future studies. In particular, we asked: what is the temporal relationship between exposure and excretion? What is the best urine collection to indicate past exposure? Approximately how quickly in 3C-NAC eliminated and is there any accumulation? Is there any evidence of kidney toxicity?

Materials and Methods

Three applicators/loaders of DCP (Telone) were studied for 3-4 days under California special registered use conditions under which maximal legal exposure might occur. Application rates were up to 12 pounds per acre in sandy dry soil and air temperatures exceeded 100°F. On any day, exposure to DCP varied from 3.5-10 hours. Urine collections were taken at each void (variable intervals 2-16 hours). Urine was collected into opague polyethylene containers (500 ml), frozen on dry ice and then stored at -70 C until analysis. Urine volumes were measured to the nearest 5 ml. In collaboration with the Worker Health and Safety Branch of the California Department of Food and Agriculture, air concentrations of DCP were measured during exposure. Operator breathing zone air samples were drawn by personal air sampling pumps (MSA Fixt-Flo) through charcoal sorbent tubes (SKC #226-09, 400/200 mg) via tygon tubing. Sorbent tubes were oriented down and affixed to workers so as not to interfere with normal work habits. Pumps were calibrated to 1 L/min using a Kurz 540S mass flow calibrator. Tubes were changed at 4 hour intervals, capped and stored on dry ice. DCP was eluted from the charcoal sorbent tubes with carbon disulfide and analyzed by electron capture gas chromatography (methods presented elsewhere or see capture gas chromatography (methods presented elsewhere or see NIOSH method 1013).

Kidney toxicity has not been reported in man and it is the most sensitive toxic effect in animals. We assumed normal clinical laboratory determinations would not be sensitive enough to indicate if renal injury were occurring (e.g. serum creatinine

determinations). The urines collected in the study were tested using N-acetyl glucoseamidase enzymatic activity (NAG). This enzyme is released from lysozomes within the tubular epithelium of the nephron (13). This marker tends to revert to normal as soon as injury is discontinued. It is considered more sensitive to this mechanism of injury than other clinical tests. The technique is described elsewhere (13). Briefly, an aliquot of urine is mixed with buffer and the ester of methyl umbelliferone and glucosamine. To the amount of methyl umbelliferone released at one hour compared to blank determinations (without urine containing enzyme) are expressed in nanomoles per hour per milligram of creatinine excreted in the urine.

Results and Discussion

The ranges of air concentrations and excretion of 3C-NAC and NAG are listed in Table II. Worker No. 3 was exposed to larger amounts of DCP towards the end of his last application. Urines following this period were not collected.

Table II. Summary Ranges of DCP Study - 1986

	Air DCP (ppb)		Urine 3C-NAC			Urine NAG (NL < 100)
Subject	Loading	Application	ug/m	ug/collection	ug/hr	nmol/hr/mgCreat
1	29-700	54-353	3.1-13.0	394-4342	90-861	47-108
2	2-335	1-19	0.9-9.1	81-607	16-211	78-678
3	16-748	65-10335	0.9-17.1	81-4363*	4-545*	25-178

*missing urine

Figure 2 shows the cumulative air DCP exposure (concentration x time) and the urinary excretion of 3C-NAC at each collection time period for a single worker. From the two workers where complete information was available, peak excretion of 3C-NAC followed peak exposure of 6-16 hours. The air concentration x time product correlates with the excretion of 3C-NAC as noted in previous studies (1), whereas concentration alone showed poor correlation. Using the five data points available from these two subjects, the best correlations with the air concentration x time were rated in the following order: cumulative ug excreted > morning spot urine 3C-NAC concentrations > interval 3C-NAC concentrations > ug 3C-NAC/mg of urinary creatinine excreted. The correlation was best when cumulative exposures (consecutive concentration x time products) were summed for two exposure periods on either side of a peak exposure versus the cumulative excretion for 3C-NAC during one period before peak excretion and two periods after peak excretion ($R = 0.961$, see Figure 3). To the best approximation, this accounts for the la exposure and excretion. To ascertain a method more useful for biomonitoring, we tried to assess whether the concentration of 3C-NAC in the morning urine following exposure correlated with the previous day's cumulative exposure (see Figure 4). This served relatively well, but more data will need to be

gathered in a systematic manner. This study was preliminary in nature and elimination kinetics were not precisely defined. It was apparent that the greater the exposure, the greater the amounts excreted. This suggested at least a pseudo first order process with half lives ranging from 2-8 hours.

Our preliminary findings on the renal enzyme (NAG) activity through testing urine samples of workers revealed a slight elevation of NAG levels. The NAG activity in these workers' urines were also higher in the morning following exposure. Since clearance of these enzymes may be dependent on flow, stasis, and other factors, the interpretation of this is not yet clear. To elucidate the possible correlation between the elevation of NAC activity and renal toxicity we need to monitor more workers in a systematic manner, and under well-controlled conditions.

These preliminary studies have been used to direct expanded field studies now underway in attempts to further describe the correlation between DCP exposure and 3C-NAC excretion, as well as definition of elimination kinetics.

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Figure 1. Structure of metabolite 3C-NAC

Figure 2. DCP exposure and 3C-NAC excretion in a single worker over 3 days.

Figure 3. Cumulative 3C-NAC excretion (sum of peak excretion period + preceding = two following periods) versus cumulative DCP exposure (sum of time x concentration products for peak period + two subsequent periods) (two workers, 5 intervals).

Figure 4. Concentration of 3C-NAC in morning spot urine versus cumulative DCP exposure in previous 24 hours (two workers, 5 intervals).

FIGURE 1

N-ACETYL-S-(CIS-3-CHLOROPROP-2-ENYL)-CYSTEINE (3C-NAC)

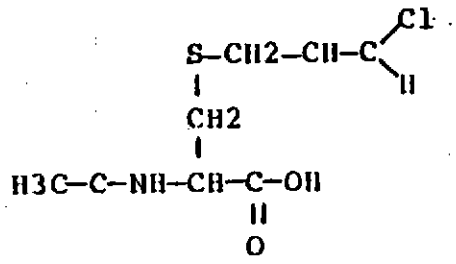


FIGURE 2

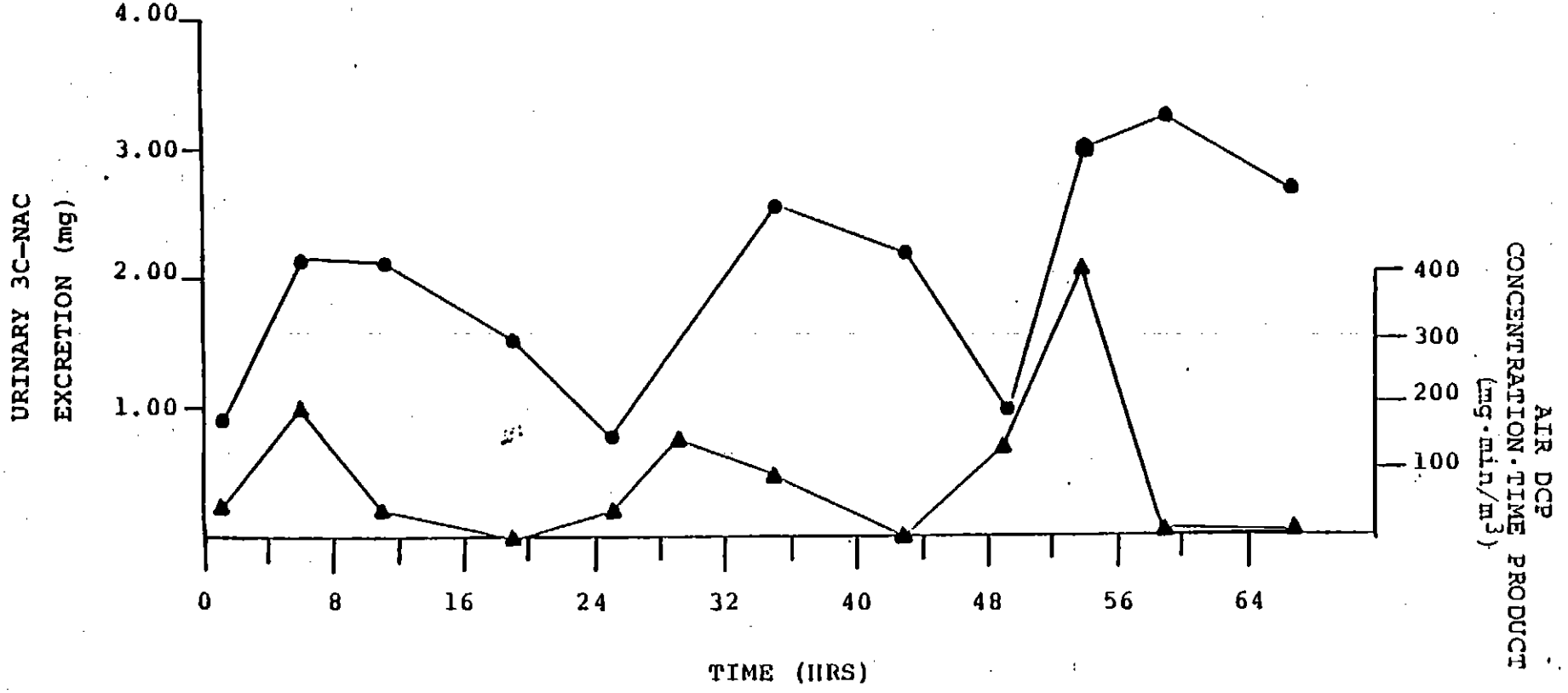

URINARY 3C-NAC EXCRETION (mg)
4.00
3.00
2.00
1.00
0
8
16
24
32
40
48
56
64
TIME (HRS)
400
300
200
100
AIR DCP CONCENTRATION-TIME PRODUCT (mg·min/m³)

FIGURE 3

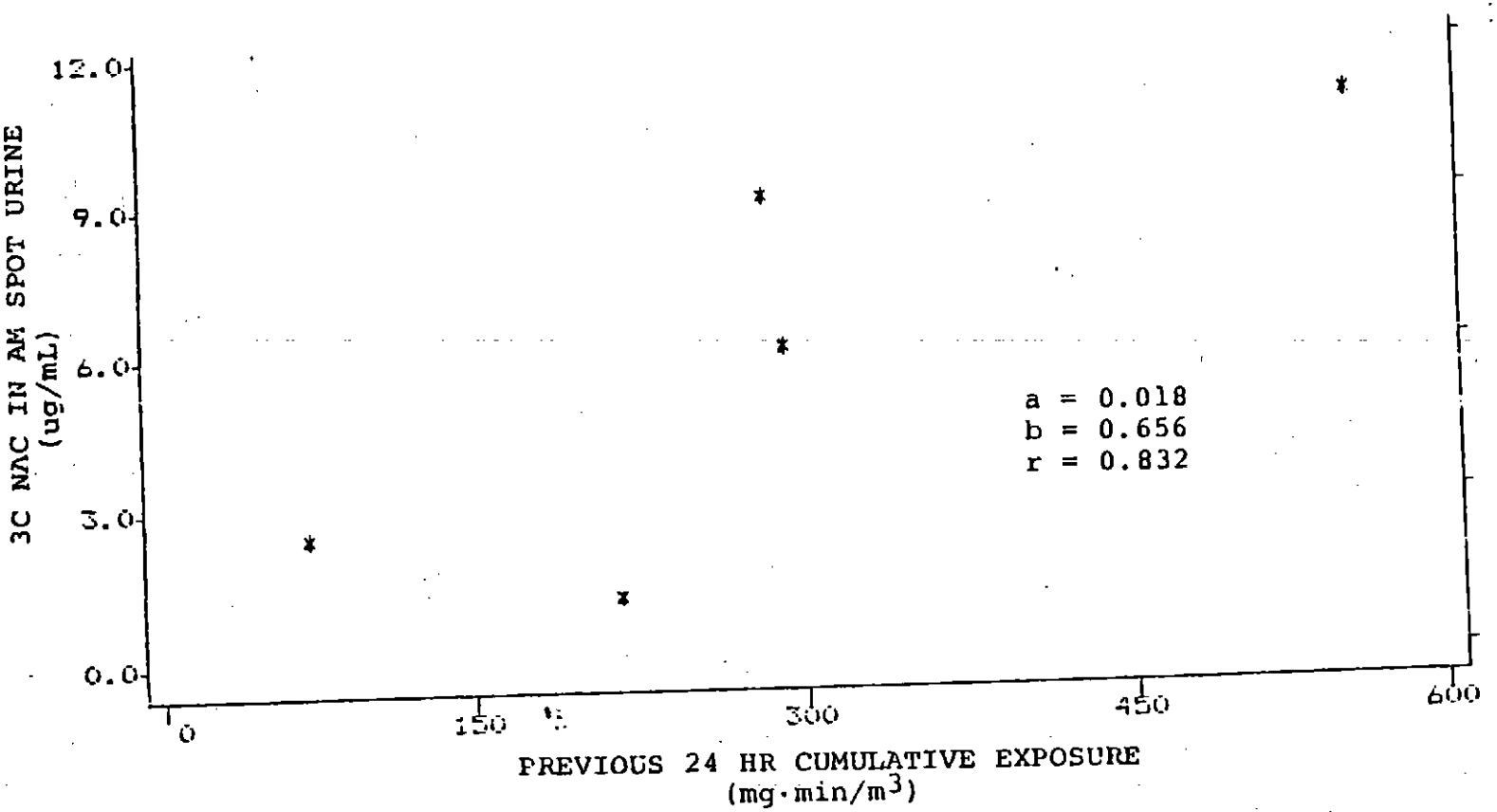
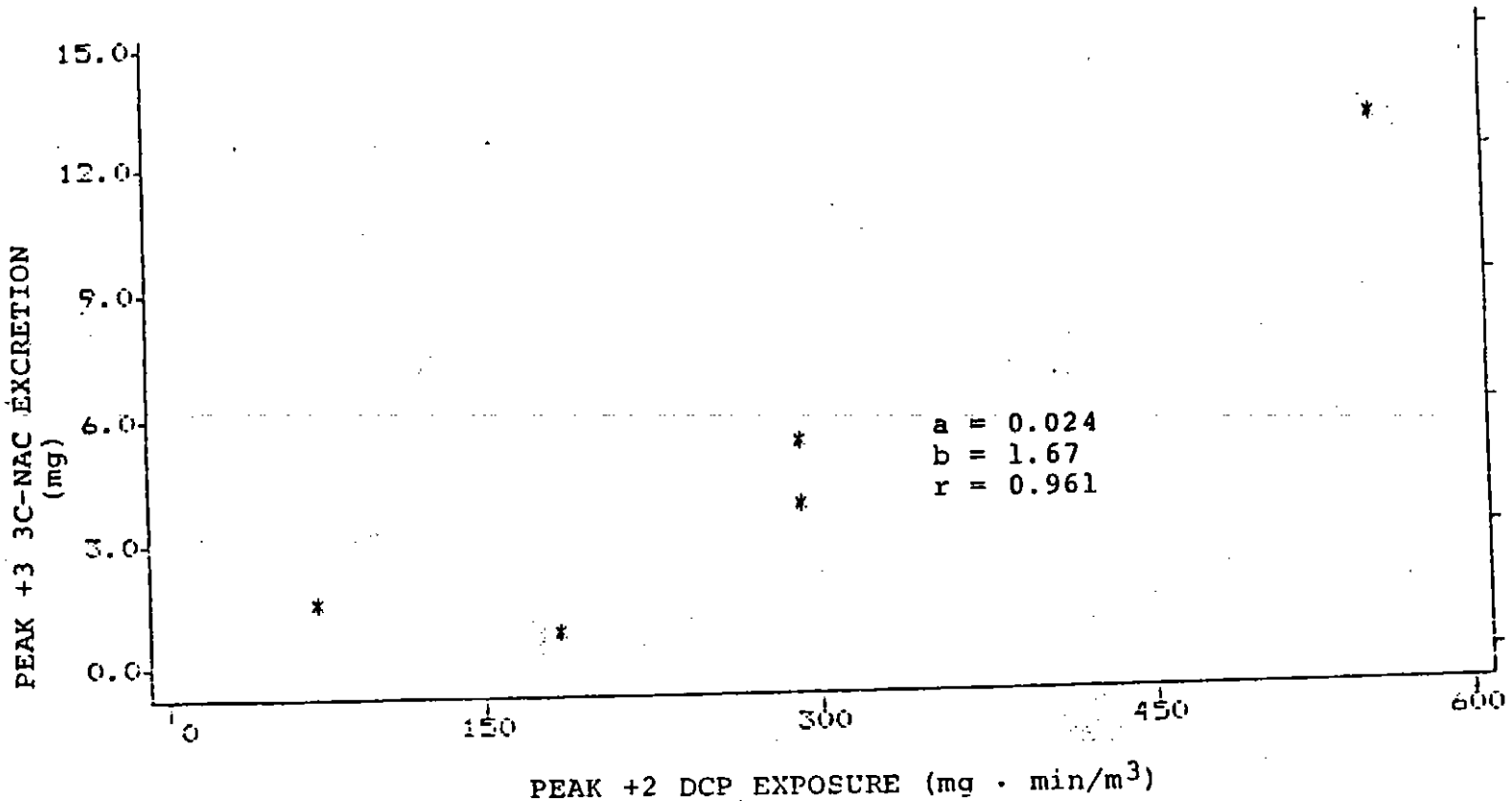

12.0
9.0
6.0
3.0
0.0
3C NAC IN AM SPOT URINE (ug/mL)
a = 0.018
b = 0.656
r = 0.832
0
150
300
450
600
PREVIOUS 24 HR CUMULATIVE EXPOSURE
(mg·min/m3)

FIGURE 4


15.0
12.0
9.0
6.0
3.0
0.0
PEAK +3 3C-NAC EXCRETION (mg)
a = 0.024
b = 1.67
r = 0.961
0
150
300
450
600
PEAK +2 DCP EXPOSURE (mg · min/m3)